The Matrix Metalloproteinase Family

J. Frederick Woessner, Jr.

Department of Biochemistry and Molecular Biology, University of Miami School of Medicine, Miami, Florida 33101

I. Introduction

Seldom has a field had such a clearly defined beginning as did the field of matrix metalloproteinases (MMPs). The first report of an enzyme from vertebrate (as opposed to bacterial) sources that was capable of attacking the triple helix of native type I collagen was published in 1962 by Jerome Gross and Charles Lapiere. In this first report they demonstrated that the enzyme activity, secreted by cultured tissue fragments of tail fin skin from resorbing tadpole tails in metamorphosis, was a true collagenase acting on collagen at 27°C at neutral pH. They also found activity produced by cultured chick embryo skin, postpartum rat uterus, and mouse and rat bone. Activity was not found in tissue extracts. In retrospect this is now known to be due both to the ability of MMPs to bind to the extracellular matrix and to the stimulation of high enzyme secretion that occurs when tissues are excised and cultured. Also in retrospect we see that at least collagenase 1 (MMP-1) and 3 (MMP-13) were present and possibly collagenase 4 (MMP-18) in these first experiments. Within a short period of time these studies had been broadened by the work of many groups to include various

human sources including neutrophils (MMP-8) and to show that collagenase cut the triple helix at a point one-quarter of the distance in from the C-terminal end and that the activity was metal dependent.

Since Gross and Lapiere's 1962 report, there has been a tremendous efflorescence of this field; in preparing for a more detailed overview I have compiled a bibliography of 8500 items, with the current rate of publication approaching 1000 items per year. The reason for this interest is pointed out most clearly by Table I, which shows some of the points of involvement of the MMPs in normal biologic and pathologic processes. Cancer and its metastatic spread have been of the greatest current widespread interest. Not only are a great many different MMPs involved in the processes of carcinogenesis and its accompanying remodeling or destruction of the extracellular matrix, but there is also

TABLE I

NORMAL AND PATHOLOGICAL PROCESSES IN WHICH MMPS ARE IMPLICATED

Normal	Pathological
Development	**Tissue Destruction**
Blastocyst implantation	Rheumatoid arthritis
Embryonic development	Osteoarthritis
Nerve growth	Cancer invasion
Growth plate cartilage removal	Cancer metastasis
Skeletal, bone growth	Decubitus ulcer
Nerve outgrowth	Gastric ulcer
Enamel maturation	Corneal ulceration
Primary tooth resorption	Periodontal disease
Reproduction	**Fibrotic Diseases**
Endometrial cycling	Liver cirrhosis
Graafian follicle rupture	Fibrotic lung disease
Luteolysis	Otosclerosis
Cervical dilatation	Atherosclerosis
Postpartum uterine involution	Multiple sclerosis
Mammary gland morphogenesis	
Mammary gland involution	
Rupture of fetal membranes	
Maintenance	**Weakening of Matrix**
Remodeling of bone	Dilated cardiomyopathy
Hair follicle cycle	Epidermolysis bullosa
Wound healing	Aortic aneurysm
Angiogenesis	
Apoptosis	
Nerve regeneration	
Macrophage function	
Neutrophil function	

hope of countering this destruction by various approaches such as systemic administration of inhibitors of MMPs, gene therapy to knock out enzymes, or overexpression of natural inhibitors such as the TIMPs (tissue inhibitors of metalloproteinases).

II. The Position of the MMPs within the Class of Metalloproteases

The proteases comprise both exopeptidases and endopeptidases (proteinases). These hydrolytic enzymes can be divided into four classes based on the catalytic group at their active center: serine/threonine, cysteine, aspartic, and metallo. It is the class of metalloproteases that concerns us here; most of its members depend on zinc for their catalytic action. A new compendium of proteases (Barrett *et al.*, 1998) describes 200 metalloproteases, of which only 14 are MMPs. Therefore, it is important to understand the relationship of the MMPs to this larger class. A useful, but still provisional, guide has been provided by Rawlings and Barrett (1995), who divide the class into clans (based on similarity of protein fold) and families (based on evolutionary relationships). Currently the metallo class comprises eight clans and some 40 families (Barrett *et al.*, 1998). For example, enzymes in clan MA have the zinc-binding motif HEXXH and the third zinc ligand is Glu. Thermolysin provides the X-ray structure for this clan and 5 families are typified by thermolysin, mycolysin, neprilysin, membrane alanyl aminopeptidase, and peptidyl-dipeptidase A (Rawlings and Barrett, 1995); there are currently 32 species of enzymes in this clan (Barrett et al., 1998). This HEXXH motif is widespread and occurs in several additional clans.

The clan containing the MMP family is clan MB in which the third zinc ligand is not Glu but rather a third His residue in the consensus sequence HEXXHXXGXXH. The two major families in this clan are M12, with its subfamilies astacin and reprolysin, and M10, with its subfamilies serralysin and matrixin (MMP). These two families contain more than 60 members and there are two further families of one member each (autolysin and snapalysin; Barrett *et al.*, 1998). The X-ray structure of this clan is now known for a number of species in the four subfamilies. Bode *et al.* (1994) have given the name *metzincins* to this group because all contain a conserved Met residue to the carboxy side of the zinc site, which produces a turn in the protein chain that provides the base of the active center binding pocket. However, the sequence similarities are not otherwise very close between families M10 and M12. The similarities of the binding pocket have the important consequence that hydroxamate inhibitors designed to block the action of MMPs are frequently found to effectively block members of the M10

family. A classic example is the inhibition of tumor necrosis factor α (TNF-α) convertase, an enzyme now known to belong to the ADAM family within the reprolysin subfamily (ADAM-17; Black *et al.*, 1997). I suspect that the "aggrecanase" activity important in cartilage degradation may also prove to be such an example.

It can be seen that less than half of the metallo class is accounted for by the first two clans. Further clans include enzymes with the HEXXH motif and a different third ligand for zinc, the motif HXXEH (pitrilysin), and a variety of additional three-ligand arrangements involving various combinations of three residues selected from His, Asp, and Glu, as well as some cases in which there are two zinc atoms and some in which the ligands remain unknown. Full details may be found in Barrett *et al.* (1998). The scheme is provisional in that we do not yet know the three-dimensional structure of many of the enzymes, and the ligands that bind zinc have not been confirmed in many cases. Note that there is no uniform practice in this field for the naming of families, so the widely used terms *MMP family* or *matrixin family* actually describe a subfamily.

An earlier set of criteria (Woessner, 1994) for assignment of a new enzyme to the matrixin family included the display of proteolytic activity, function outside the cell, possession of conserved sequence around cysteine in the propeptide (PRCGxPD) and a zinc-binding consensus sequence of HEXGHXXGXXHS/T. However, members of family 10 also meet all of these criteria except the last. Today, a better criterion would be a cDNA sequence that is sufficiently close to that of collagenase to permit assignment to the matrixin subfamily. Many synthetic inhibitors of MMPs, particularly in the hydroxamate series, inhibit members of family 10 equally well as noted earlier. However, TIMP-1 does not appear to block any members of that family, but does appear to inhibit all MMPs. This provides a second criterion.

III. The Matrixin Subfamily

A. *Members of the Subfamily*

At the time of this writing (late 1997), 17 enzymes have received MMP numbers. The numbers are not being assigned by an official governing body, so some confusion arises as individual authors assign numbers they believe come next (e.g., Cossins *et al.*, 1996). It was suggested at the 1997 Gordon conference on MMPs that I make the assignments in the future. This plan is feasible only if the new enzymes are discovered by those already working in the field. The MMP numbers are often convenient to use as a shorthand when speaking or writing,

but their utility is somewhat diminished by the large number of enzyme species that are coming to light. Table II indicates the most commonly used names, including those recommended by the International Union of Biochemistry and Molecular Biology (through MMP-12). Three enzymes reported earlier (MMP-4, -5, -6) were later found to correspond to known enzymes, so these three numbers have been discontinued and remain vacant. The enzymes are placed into arbitrary groups that originally arose from considerations of the substrates cleaved. This is not a very sound basis, because we have very little information about the natural substrates of any of these enzymes (see later discussion). Stromelysin 3, while having some substrates in common with the other two stromelysins, is very distantly related and is activated through a furin site and only indirectly through disruption of the cysteine switch. The membrane-type MMPs have been grouped on the basis of possession of a transmembrane domain. Table II also notes a few of the many synonyms for these enzymes and indicates additional features of some MMPs.

B. *Evolutionary Relationships among the MMPs*

A dendrogram showing the relationships among the MMPs known from humans is presented in Fig. 1. The figure is limited to human enzymes because the more than 65 known sequences from all species produce a tree that is difficult to see for the forest of entries. Only the approximately 170 residues of the catalytic domain for each enzyme have been used in deriving this tree. The earliest branches include MMP-19 and the four membrane-type MMPs. These MT-MMPs form a tight cluster except for MT4-MMP, which is somewhat earlier than the others. A metalloproteinase of the human ovary has been reported as a gene sequence (Acc. No. D83647), but nothing is known of its properties. As alluded to earlier, stromelysin 3 arose quite early relative to the other two stromelysins. Stromelysin 3 and the four MT-MMPs have in common the RXKR/RRKR furin cleavage site, suggesting that they may be activated while still in the cell, whereas the remaining enzymes require proteolytic cleavage of their propeptide after the zymogen leaves the cell. It is interesting to speculate that simpler organisms regulated their matrix largely by contact, through cell surface MMPs; then as more extensive and complex extracellular matrices evolved, it became advantageous to secrete the MMPs for action at a distance from the cell.

The last 10 enzymes form a cluster of the more modern and more commonly known MMPs. The two gelatinases are first in this group; they contain additional fibronectin-like domains. Matrilysin, the small-

TABLE II

MEMBERS OF THE MATRIXIN FAMILY

Group name	MMP number	EC number	M_r latent/active	Notes
Collagenase				
Collagenase 1	MMP-1	EC 3.4.24.7	52,000 42,000	**Interstitial collagenase**
Collagenase 2	MMP-8	EC 3.4.24.34	85,000 64,000	**Neutrophil collagenase**
Collagenase 3	MMP-13		52,000 42,000	Rodent interstitial collagenase
Collagenase 4	MMP-18		53,000 42,000	*Xenopus*
Gelatinase				
Gelatinase A	MMP-2	EC 3.4.24.24	72,000 66,000	Type IV collagenase
Gelatinase B	MMP-9	EC 3.4.24.25	92,000 84,000	Type V collagenase
Stromelysin				
Stromelysin 1	MMP-3	EC 3.4.24.17	57,000 45,000	Transin
Stromelysin 2	MMP-10	EC 3.4.24.22	54,000 44,000	Transin-2
Stromelysin 3[1]	MMP-11	EC 3.4.24.	64,000 46,000	RXKR furin cleavage
Membrane-type				
MT1-MMP	MMP-14		66,000 54,000	Transmembrane domain and RRKR furin cleavage site
MT2-MMP	MMP-15		72,000 60,000	
MT3-MMP	MMP-16		64,000 53,000	
MT4-MMP	MMP-17		57,000 53,000	
Others				
Matrilysin	MMP-7	EC 3.4.25.33	28,000 19,000	Lacks hemopexin
Metalloelastase	MMP-12	EC 3.4.24.65	54,000 22,000	**Macrophage elastase**
(No trivial name)[2]	MMP-19		54,000 45,000	
Enamelysin[3]	MMP-20		54,000 22,000	
Nonmammalian				
Xenopus XMMP[4]			70,000 53,000	Cys in catalytic domain
Envelysin[5]			63,000 48,000	Sea urchin
Soybean MMP[6]			? 19,000	Protein sequencing

Note: The values of M_r, except for MMP-8, are based on cDNA sequence; glycosylation may increase these values. Values for the active forms of MT-MMPs assume cleavage at the furin site. Names in bold are those recommended by the IUBMB. Certain of these enzymes do not receive further attention in the individual chapters; reference to these is as follows: [1] Basset *et al.*, 1990; [2] Cossins *et al.*, 1996; Pendas *et al.*, 1997; [3] Bartlett *et al.*, 1996; [4] Yang *et al.*, 1997; [5] Lepage and Gache, 1990; [6] McGeehan *et al.*, 1992.

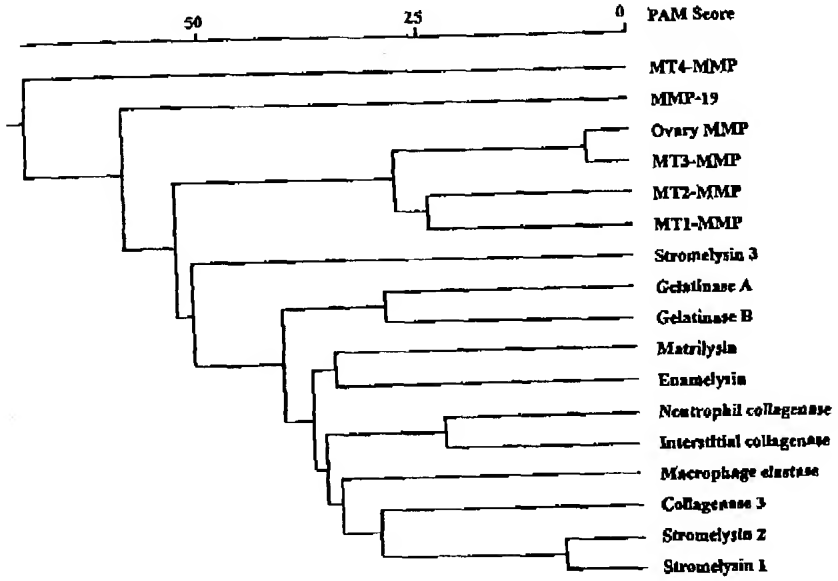


FIG. 1. Dendrogram illustrating the evolutionary relationships among the 17 known matrix metalloproteinases found to date in humans. The sequences were aligned by using the PILEUP program for the catalytic domain only (exclusive of fibronectin repeats). The tree was generated by the KITSCH algorithm. (Figure kindly provided by Dr. Neil Rawlings.)

est MMP, appears next; the absence of a hemopexin-like domain in this enzyme is probably due to a deletion, rather than to an evolutionary origin prior to the addition of hemopexin. Collagenases 1 and 2 are fairly close, followed shortly by macrophage elastase; stromelysins 1 and 2 come last. The last 7 enzymes are closely related in their domain structure, although macrophage elastase loses its hemopexin domain upon activation.

IV. The Domain Structure of the Matrixins

The matrixins form an interesting group of enzymes in that there is a central catalytic domain to which have been added a variety of additional domains or short inserts. Matrilysin represents the "minimal" enzyme—it consists of a signal peptide, a propeptide, and the catalytic domain. No one enzyme has all of the possible building blocks. If one examines the MMPs starting from the N terminus, the following features are seen:

Signal Peptide This is typically a stretch of 17–20 residues, rich in hydrophobic amino acids, that serves as a signal for secretion into the endoplasmic reticulum for eventual export from the cell. All of the MMPs except for MMP-17 (Puente *et al.*, 1996) possess a signal peptide.

Propeptide This region contains about 80 amino acids, typically with an N-terminal hydrophobic residue. There is a highly conserved PRCXXPD sequence near the C-terminal end of this segment; this provides the cysteine residue that makes contact with the catalytic zinc atom and maintains the enzyme in its zymogen form. This cysteine is found in all MMPs, including those that have the furin-cleavage site.

Furin-Cleavage Site Insert This stretch of about nine residues includes the consensus sequence of RXKR/RRKR that leads to intracellular cleavage by furin. MMP-11, -14, -15, -16, and -17 possess this sequence. In the remaining enzymes, a cleavage by external proteases occurs in the middle of the propeptide, partially exposing the zinc and leading to autolytic cleavage of the remainder of the propeptide. The exact site of final cleavage may vary within a given enzyme leading to different degrees of activation, particularly in the case of MMP-1. XMMP contains a much longer insert of 37 residues (similar in sequence to vitronectin) that ends in the RRKR motif (Yang *et al.*, 1997).

Catalytic Domain This domain typically contains about 160–170 residues, including sites for the binding of calcium ions and the structural zinc atom. The 50–54 residues at the C-terminal end of the catalytic domain include the site of binding of the catalytic zinc. This involves the highly conserved HEXGHXXGXXHS/T sequence mentioned earlier. MMP-17, however, has Val in place of Ser (Puente *et al.*, 1996). The zinc-binding region is somewhat independent of the remainder of the catalytic domain because various insertions can occur between these two portions.

Fibronectin-Like Repeats There are three repeats of the fibronectin type II domain in MMP-2 and MMP-9, inserted in the catalytic domain just ahead of the 50-residue zinc-binding region. These specialized structures aid the binding of enzyme to gelatin substrates.

Hinge Region The catalytic domain is connected to the following hemopexin domain by a linker region usually referred to as the hinge region. It ranges in length from 0 to 75 residues. The longest hinge is found in MMP-9 and shows considerable homology to type V collagen in that it is rich in proline. MMP-7, having no hemopexin domain, has no need for a hinge and XMMP also lacks this insert. A typical hinge contains about 16 residues including a number of proline residues. MMP-19 has a highly acidic region DEEEEETE within its linker (Cossins *et al.*, 1996; Pendas *et al.*, 1997).

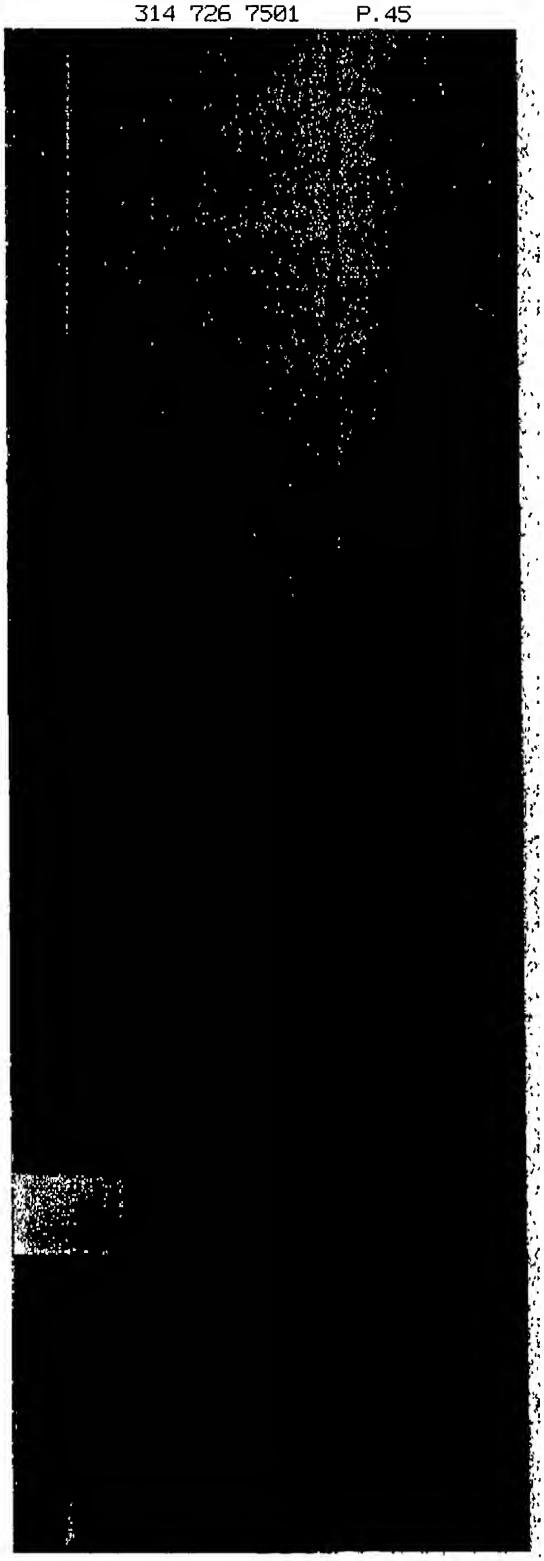
Hemopexin Domain This domain of about 200 residues contains four repeats that resemble hemopexin and vitronectin. There is a Cys residue at either end; these join and the resultant domain folds into a four-bladed propeller structure. All MMPs except MMP-7 contain this structure, but it does not appear to be essential for catalytic activity. Many truncated forms of MMPs have been produced that lack this domain and all retain activity. However, substrate specificity for macromolecules may be greatly affected (see review by Murphy and Knäuper, 1997). The binding of TIMP is also assisted by this domain and, in the case of gelatinases, there is binding of TIMP to this domain even when the enzymes are in their zymogen form.

Membrane Insertion Extension Although most MMPs have their C terminus at the end of the hemopexin domain, the four MT-MMPs have a further extension that governs insertion of these proteases into the cell membrane. Its length ranges from about 80 to 110 residues. The membrane-spanning region of about 20 residues is about 20 residues in from the C terminus, leaving this short segment within the cytoplasm. MMP-19 has a 36-residue extension beyond the hemopexin domain but this is not membrane inserted.

V. The Biological Role of the Matrixins

A. *Do the MMPs Play a Major Role in Degrading the Extracellular Matrix?*

Table I provides a good overview of the vast number of biologic and pathologic processes in which it is believed the MMPs play an important, or even indispensable, role. However, it must be admitted that firm proof for such involvement is very sparse. The general sorts of proofs that are offered include the use of *in situ* hybridization to show that mRNA for a given MMP is present at the site of tissue remodeling, use of immunohistochemistry to demonstrate that the enzyme protein is present, and localization of specific degradation products at the site (e.g., the cleaved fragments of collagen). These criteria, however, merely indicate guilt by association; they do not generally prove that a specific MMP is responsible for a specific effect. Now that we know of 17 MMPs in humans, it must be admitted that no one has examined a particular case of matrix remodeling for each activity nor established the contribution of each enzyme to the process. Although one can demonstrate the cleavage of collagen type II in arthritic cartilage (Dodge *et al.*, 1991), for example, we now know that the identical specific cleavage can be produced by MMP-1, MMP-2, MMP-8, MMP-13, and MMP-14 and there is some evidence that each of these enzymes can be produced by chondrocytes.



Krane (1994) has suggested criteria for proving the role of an MMP in a remodeling process:

- Remodeling can be blocked with a drug or antibody specific to the MMP.
- Remodeling can be reproduced by overexpression of the MMP gene in transgenic animals.
- Remodeling can be abolished by deleting the MMP gene.
- Spontaneous mutations can be identified and the phenotypes characterized.
- Mutations can be induced in the gene that reproduces the remodeling process.

Progress in carrying out this program of establishing the roles of the various MMPs has been slow and somewhat disappointing. Shapiro (1997) has recently reviewed the results with transgenic mice—there are knock-outs for MMP-3, MMP-7, MMP-9 and MMP-12 and overexpressers of MMP-1 and MMP3. In most cases these have not led to sharply defined phenotypes and they have not led to death of the organism. A problem appears to be the high redundancy of function of the MMPs, so that when one enzyme is knocked out another becomes more highly expressed to compensate for the loss. However, Shapiro has established quite clearly that the mouse macrophage, with a somewhat limited repertoire of MMPs, requires MMP-12 (macrophage elastase) in order to produce emphysema in mice induced to inhale cigarette smoke (Hautamaki *et al.*, 1997). A similar problem arises in the use of specific inhibitors to block the action of an MMP. In general, the active centers and bond specificity of the various MMPs are fairly similar. For example, the collagenase substrate DNP–Pro–Leu–Gly*Ile–Ala–Gly–Pro–D–Arg is cleaved by all the MMPs tested to date. Furthermore, MMP inhibitors currently in use such as Batimastat are hydroxamate compounds; these are found to inhibit members of the M10 family, such as TNF-α convertase, as well as MMPs (Black *et al.*, 1997). However, progress is being made in making such inhibitors ever more specific as can be seen in Chapters 10 and 11.

B. What Are the True Substrates for Each MMP?

It is very common to find reviews containing a table similar to Table II in which there is a list of substrates for each MMP. A still more extensive compendium of substrates is provided by Chandler *et al.* (1997). However, I have avoided this practice because it is my opinion that almost nothing is known about the natural substrates of the MMPs and that such a table is very misleading. Investigators tend to test

only those matrix components ready to hand. Because many are cell biologists, their shelves contain collagens I and IV, fibronectin, laminin, and nidogen. However, I estimate that there are more than 100 known macromolecular components of the extracellular matrix—about 30–40 each of collagens, proteoglycans, and glycoproteins. Examination of the digestion of this vast array of potential substrates by the 15 MMPs is barely under way.

To establish a natural enzyme–substrate relationship, it is necessary to show that the substrate is cleaved *in vivo* at a certain point that matches the specificity of the enzyme and is unlikely to be due to other proteases. The case on which the greatest attention has been lavished is that of the cleavage of the large aggregating proteoglycan aggrecan, found in articular cartilage. A good deal of circumstantial evidence was built up by showing that stromelysin 1 could readily digest the protein core of aggrecan and that stromelysin was elevated in osteoarthritic cartilage (Dean *et al.*, 1989). However, more detailed study of the cleavage of aggrecan by various MMPs showed that every one of those tested, including collagenase 1, 2, and 3, could produce the same specific cleavage at the bond DIPEN*FFGVG (Fosang *et al.*, 1996). Moreover, when aggrecan degradation products were isolated from cartilage and synovial fluid, the products had arisen from the cleavage of an ITEGE*ARGSV bond (Sandy *et al.*, 1992). It therefore appears that a novel protease, aggrecanase, is required for this cleavage and that this is a metalloprotease but probably not an MMP.

This case is sufficient to illustrate the problem. The redundancy of specificity of the MMPs makes it difficult to pin down which one did what, and very few cases have had the cleavage sites of the substrate examined *in vivo*. Even in a clear case such as the interstitial collagens, which show the specific cleavage by collagenases of their Gly–Ile bond, a cleavage unlikely to be produced by other proteases in the tissue, one is left with five contenders for the role of cleaving enzyme. However, in the transgenic mouse in which this collagen cleavage site is mutated, collagen is still degraded through cleavage of the telopeptides. In this case, MMP-13 appears to be the enzyme that is capable of this cleavage (Krane *et al.*, 1996).

C. *Why So Many MMPs?*

In view of the redundancy of function of many of the MMPs, one may ask why so many enzymes have evolved. In spite of the difficulty of proving what each one does, I believe that the multiplicity of forms underlines the extreme importance of the MMPs for the normal morphogenesis, maintenance, and repair of the matrix. The individual

cell is very highly dependent on its battery of MMPs to control its environment, to move through it, and to maintain its protective cocoon. The knock-outs seem to tell us that the cell can make do without one of these enzymes by mobilizing one or more of the remaining MMPs to take over the function of the deficient enzyme. Furthermore, we should not think that each cell possesses the entire battery of enzymes. So far as is known, enamelysin (MMP-20; Bartlett *et al.*, 1996) is found only in developing tooth enamel. MMP-8 and MMP-12 are largely confined to the neutrophil and macrophage, respectively. MMP-7 appears to be restricted to epithelial cells (Wilson and Matrisian, 1996). The MMPs must be considered one more example of the profligacy of nature.

VI. What the Future Might Hold

The imminent arrival of the millenium prompts one to prognosticate about the prospects for the MMP field. Activity in this area of research has now reached a fever pitch and, with the clear involvement of the MMPs in such major disease problems as cancer, arthritis, and atherosclerosis, it seems unlikely that a sudden decline in interest in the MMPs will occur. Diseases will remain a major driving force because economics and politics will favor the distribution of resources in that area. Current work on the development of specific inhibitors will intensify. Both the working out of the detailed specificity requirements of each MMP and the development of highly specific inhibitors will be facilitated by the increasing use of combinatorial chemistry. However, there appear to be limits to the specificity that might be achieved, with consequent unintended side effects, so increasing attention will be focused on regulating MMP activity through genetic techniques such as antisense methods and through the use of specific drugs/factors that can regulate the expression of each MMP. This will require more detailed knowledge of promoters and cell factors governing expression. Intervention may also be attempted at the level of proteolytic activation of the MMP zymogens.

With respect to fundamental problems that remain to be answered, I am interested in the binding of MMPs to the cell and substratum. It is very difficult to extract most of the MMPs from tissues due to various types of anchoring. It is probably crucial for the cell to keep the MMPs in its vicinity to govern their activity, to keep track of how much enzyme is out there, and to prevent the enzymes from washing away with the blood until needed. A number of the MMPs appear to be attached to the cell surface bound to receptors, inserted into the membrane, or localized to invadopodia. This permits the cell to effect proteolysis in a specific direction following a regulated process of surface activation.

Much more needs to be learned about how the cell senses the proteolytic activity in the outside world—through receptors, integrins, or similar signaling mechanisms and through feedback from substrate fragments interacting with the cell. We need to learn more about how the cell regulates the activation of MMPs already released from the cell. Finally, with the detailed structure of MMP–TIMP complexes now in hand, we can begin to explore in more detail how the TIMPs interact with the enzymes. The full story here can only emerge when we see the full structure of the gelatinases with TIMPs in place on both the proenzyme and the active enzyme.

In summary, the past 36 years have seen tremendous advances in our understanding of the structure and activity of the MMPs. However, the areas of ignorance appear to be almost infinite in extent, promising many exciting years ahead in the MMP field.

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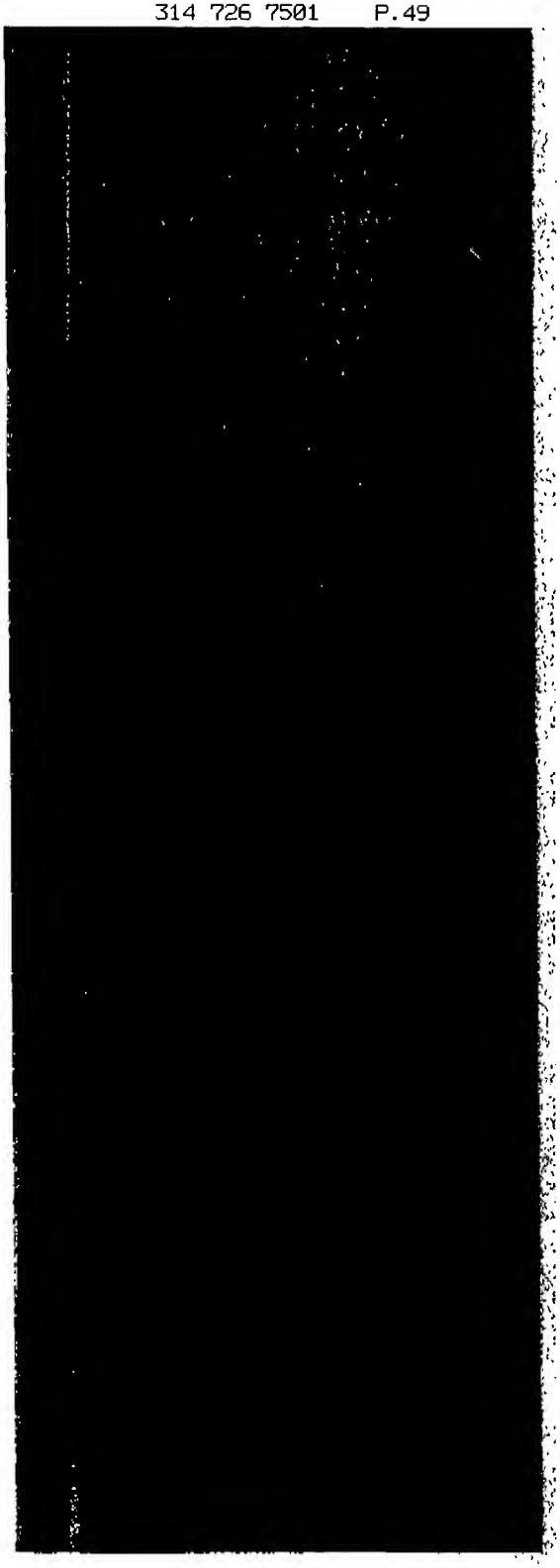

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